1977

The Effects of Superovulation on the Reproductive Cycle of Mature Female Mus musculus

David F. Grace
Eastern Illinois University

PAPER CERTIFICATE #2

TO: Graduate Degree Candidates who have written formal theses.

SUBJECT: Permission to reproduce theses.

The University Library is receiving a number of requests from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow theses to be copied.

Please sign one of the following statements:

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

8-5-77
Date

Author

I respectfully request Booth Library of Eastern Illinois University not allow my thesis be reproduced because_____

_____

_____

_____
Date

_____
Author

pdm

The Effects of Superovulation on the Reproductive Cycle of Mature Female Mus Musculus

(TITLE)

BY

David F. Grace

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1977
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

8-3-77
DATE

ADVISER

8-3-77
DATE

DEPARTMENT HEAD

ABSTRACT

A total of 38 Mus musculus were used to compare superovulation to normal ovulation in three different areas of the reproductive cycle. The areas studied include the fertilization and abortion rates, alternation of ovarian function, and normalization of estrus cycling. No statistically significant differences were noted between the two groups in either fertility rates or abortion rates. A distinct difference was noted in alternation of ovarian function. Normally ovulating mice showed a distinct right or left preference in the number of ova ovulated from each ovary. Animals showing a left preference exhibited approximately a 2:1 ratio and animals with a right preference exhibited approximately a 3:1 ratio. Superovulated mice showed no preference and exhibited approximately a 1:1 ratio of left to right ova ovulated. A significant difference was also noted in normalization of cycling following mating. There was no significant difference between the normalization rate following normal ovulation as opposed to superovulation treatment. However, when mating occured, a significantly longer time span elapsed before cycling resumed.

ACKNOWLEDGEMENTS

I would like to extend to Dr. Eugene Krehbiel my respect and gratitude for helping me in the research and development of this paper. Special thanks also go to Mr. Tom Beissel for preparing serial sections.

TABLE OF CONTENTS

LIST OF FIGURES

Introduction

Superovulation is the process of inducing ovaries to ovulate more than the normal number of ova. In mice eight ova are normally ovulated but with 40 IU Pregnant Mares Serum (PMS) and 40 IU Human Chorionic Gonadotropin (HCG) from 14 to 48 ova are ovulated (Sato 1962). Rabbits normally ovulate seven or eight ova but with superovulation using relatively pure Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Maurer, Hunt, and Foote,1968) can ovulate about 47 ova. Hamsters normally ovulate ten ova, but with treatment with PMS and HCG (Greenwald, 1962), ovulate up to 74 ova.

There are two methods of superovulation, X-irradiation of the ovaries and through the use of hormones. In Long-Evans rats, X-irradiation of the ovaries resulted in approximately the same number or slightly more than normal ova being ovulated. According to Fengols and Hahn (1972), prefertilization and x-irradiation induced exposure dependent increases in ovulation, implantations and live term fetuses. At 200r the number of ovulations increased and at 400r, both the number ovulated and the fertilization rate decreased. Mandl (1964) reported the greatest number of ova ovulated with unilateral irradiation with 157r and exposure of the other ovary to air (surgical handling). Irradiation with 315r to 630r caused a decrease in the number of ova ovulated. Superovulation by irradiation is apparently due to non-specific damage to the ovary mediated by a reduction of the number of pre-existing large graffian follicles which become atretic. Irradiation

is not believed to cause maturation of the "reserve follicles". With larger doses (greater than 315r) the ovary treated either fails to ovulate any ova or ovulates a reduced number; the other ovary, however, exhibits a compensatory effect and produces the number of ova normal for that animal. Surgical removal of one ovary has the same effect on the number of ovulated ova (Weifenbach, 1965).

The second method of superovulation is through the use of hormones. A follicle "priming" hormone (FSH) is given followed 48 to 72 hours later by an "ovulatory" hormone (LH) (Gates, 1971). PMS is the most commonly used "priming" hormone and HCG is the most commonly used "ovulatory" hormone. Superovulation is believed to occur as a result of changes in each ovary causing maturation and development of pre-existing follicles and "reserve follicles" which equals a maximum for superovulation in that organism. According to Hafez (1969), there are quantitative and qualitative differences in the responses of animals to crude and purified PMSG. In some species, refractoriness to repeated injections also occurs. These responses vary with season, breed, weight, stage of estrous cycle and individual variations in the cycle length, age, genetic constitution, amount of hormone administered previously, postpartum interval, and state of nutrition.

One study on seasonal changes in superovulation by Moore and Greenwald (1974) in hamsters indicates a significant increase in the number of ova ovulated with superovulation treatment in the months of June, December, and January. According to Hafez (1964) there was no significant seasonal differences in the number of ova ovulated in domestic rabbit with or without superovulation. However, he noted that

the number of ova seemed higher in January, February, and March than in October, November, and December.

Different breeds of animals react differently to superovulation mainly in the number of ova ovulated. In a study of three different breeds of mice by Schneider and Hahn (1975) the percent of ova developing after superovulation ranged from 0% in one breed to 57.9% in another. There was also a difference noted in the fertilization rates of the three strains studied. An increase in the ovulation rate led to a decrease in the fertilization rate.

The age and weight of an animal are of primary importance in considering superovulation and its results (Gates, 1971). Prior to puberty an animal can produce, with superovulation, a large number of ova but is incapable of maintaining those ova. After puberty the animal produces and is capable of maintaining a fairly constant number of ova. However, as the animal approaches "old age" the number of ova decreases until the animal no longer ovulates (Maurer and Foote, 1971). If the weight of an animal is retarded due to diet or development in a large litter (Wilson, Runner, and Zarrow, 1963) the relative ovarian weight is depressed. Animals which are retarded as evidenced by lowered body weight tend to yield reduced numbers of ova (Lang and Lamond, 1966).

The effects of superovulation at different stages of the estrous cycle have been studied in depth in the hamster. The hamster has a four day estrous cycle, and according to Greenwald (1962) if given 30 iu PMS on day 1 (metestrus) between 9 a. m. and 10 a. m., about 70 ova will be ovulated on day 4 as compared to 10 ova normally ovulated. If injected on day 2, the hamster will not ovulate until day 6 and will only ovulate

about 50 ova. The ten pre-existing large graffian follicles from each ovary become atretic and only reserve follicles are matured and ovulated. If injected on day 3, 50 ova are obtained but ovulation does not occur until day 7. Injection of PMS made on day 4 results in the ovulation of the normal number of ova on that day and ovulation of 50 reserve follicles at the end of the next cycle. In a study by Ikeda and Sakuma (1974), PMS increased the rate of pregnancy when given to hamsters on days 1 or 2. PMS injections also yielded a larger percentage of animals copulating. All animals receiving PMS on days 1 and 2, and those receiving from 10 to 30 iu PMS on day 3 copulated. Those receiving less than 5 iu in day 3 and all doses on day 4 did not copulate. Superovulation also affects the losses during gestation. In a study by Maurer, Hunt, and Foote (1968) about half of the ova in superovulated mice were lost prior to implantation of the embryos as compared to only a 10% loss in the normally ovulated mice. Shortly after implantation, another 13% to 18% degenerate in the superovulated mice as compared to a 3% loss in the controls. Another 41% of the remaining superovulated embryos died or were in the "mole" stage at mid-pregnancy as compared to 10% in the controls. It was also noted in this article that the litter size of superovulated mice carrying full term were smaller than the normal litter size due to high fetal mortality during pregnancy.

Most of the work with superovulation in mice has been done with immature mice-hence no estrous cycle. Mature mice have a polyestrous cycle with a variable duration of time between stages so when using mature mice the cycle can be synchronized. PMS given intraperitoneally in doses of 1 to 2 IU followed 48 hours later by 1 to 2 IU HCG synchronizes

the estrous cycles without superovulation (Biggers, Whitten, and Whittingham,1971).

Hormones play a major role in superovulation and their effects on it are many. In a study by Zarrow and Quinn (1963) it was shown in treating 28 day old immature rats that 30 iu of PMS alone, if synchronized with estrous cycle, caused the ovulation of 70 ova while injections of PMS and HCG ovulated a maximum of 60 ova. This seems to indicate that PMS is the most efficient means of superovulation if given at the correct stage of estrous. It should be noted that HCG is necessary when the cycle is not synchronized or when the endogenous release of LH is not sufficient to cause ovulation. Ovulation can be blocked, even after PMS treatment, by hypophysectomy or injections of dibenamine, SKF 501, atrophine, or Nembutol (Zarrow and Quinn,1963).

Anti-PMS serum also affects superovulation (Greenwald,1963). If the injection of anti-PMS serum is followed by PMS treatment on day 1, 2, or 3 of the estrous cycle in hamsters, it results in normal ovulation of only 10 ova on day 5 but no superovulation. This seems to indicate that in order for the reserve follicles to develop and the 10 large pre-existing follicles not to become atritic, there must be an adequate circulating level of PMS throughout the estrous cycle. If anti-PMS is given on day 4, no ova will be ovulated (Greenwald,1963). This seems to indicate that the follicles are dependent on PMS until ovulation.

Many other hormones influence ovulation or the various stages of embryonic development. Estradiol has been studied and shown to increase the number of ova ovulated when used in conjunction with regular

superovulation treatment in the hamster (Ishijima, Mihata, Hirabayashi, and Sakuma 1971) and in the immature rat (Wyss and Pincus, 1964). Estradiol is able to increase the number of ova ovulated if given during the first 30 hours after the PMS injection. Progesterone was shown to have no effect on the number of ova ovulated by superovulation (Wyss and Pincus, 1964). Prolactin has been shown to increase implantation of fertilized ova in mice (Sakuma and Endo, 1970). In the rabbit, it has been shown to increase the number of animals with implantation sites and the number undergoing parturition (Ishijima, Ito, Hirabayashi, and Sakuma, 1971). In another study, Ishijima, Kawase, and Hirobayashi (1971) showed that superovulation increased the rate of transport of ova into the ovaduct and sped up the rate of cleavage. The cleavage in superovulated animals occurred about 3 hours ahead of the normally ovulating animals at the 4-16 celled stages. Beaumont and Smith (1975) reported increased pre and post-implantation mortality following superovulation in mice.

Superovulation also has long term effects on the animal. Maurer, Hunt, and Foote (1968) reported repeated superovulation resulted in a decreased response to superovulation treatment. Superovulation also increases the number of individuals with chromosomal abnormalities (Fujimoto, Pahlovan, and Dukelow, 1974). In a study of 108 ova 9.7% of superovulated ova contained chromosomal abnormalities with no abnormalities detected in control ova. Several other long term effects of superovulation have also been suggested. In a study by Chretien (1972) the presence of adhesions attaching the cornua uteri together or to neighboring organs were noted in superovulated rats. In addition,

a large portion of the females treated became sterile even though spermatozoids were found in vaginal smears. In a histological study on superovulated rabbit does, Ishijima and Ishida (1970) noted abnormal follicles, congested blood vessels around these follicles, and in the interstitium, Fe-laden histiocytes. The abnormal follicles consisted of: hemorrhagic cystic, hemorrhagic leuteinizing, and hemorrhagic atretic follicles. In several of the studies quoted before, there have also been a large number of CLA (Corpus leuteum accessoria) or follicles which failed to erupt or ovulate. A few CLA were noted in control animals but a large increase of these were found in superovulated animals. This is thought to be due to insufficient endogenous LH to ovulate the increased number or mature graffian follicles ready for ovulation. This was noted more specifically in a study by Beaumont and Smith (1975).

The purpose of this study is to observe the effect of superovulation on the reproductive cycle of mature female Mus musculus. This study will consider three main areas of the reproductive cycle. The first area studied will consider the fertility and abortion rate of normally ovulated and superovulated mice. The second will be concerned with any alternation of ovarian function. And finally the third area of study will consider the rate of normalization or resumption of the estrus cycle following treatment and/or partruition.

Methods and Materials

In this study, 38 mature female Mus musculus between two and four months of age were studied to determine any significant differences in reproduction caused by superovulation. The mice were grouped five to a cage to avoid crowding and unnecessary stress and were identified by visible differences in coloration or markings. All animals were housed at approximately 68°F with constant 14-10 hour day-night periods. Food and water was supplied ad libidum. The stage of the estrus cycle was determined by vaginal lavage using a method described by Rugh (1968). Eight groups of animals were used. Each group consisted of five mice except IV and VII with four each.

The estrus cycles of animals in Group I were followed for two complete cycles and the animals were introduced to males when entering their third estrus. Fertilization was determined by the presence of the copulation plug or failure to resume cycling after 14 days. The date of resumption of the estrus cycle was noted in animals that failed to conceive. Pregnant animals were allowed to parturate and the pups were counted and sexed according to Rugh (1968). The female was then sacrificed and the ovaries removed for histological study. The ovaries were serial sectioned and stained using Toluidine Blue. Corpora lutea were counted to determine the number of ova ovulated from each ovary.

In Groups II, III, and V the same procedure was followed with the exception that upon entering their third estrus period, the five animals in each of these groups were superovulated using a method

described by Gates (1971). They were then introduced to males and the procedures again coincided with Group I.

To determine what effects parts of the superovulation treatment have on the estrus cycle - two other groups of animals were treated. Group IV, consisting of four animals, was treated with 10 iu of PMS. No injection of HCG or mating occurred. These animals were then cycled until there was a resumption of the estrus cycle. Group VI, consisting of five animals, was injected with 10 iu PMS and 10 iu HCG but were not put with males. These animals were also cycled until there was a resumption of the estrus cycle.

In order to use a larger number of ovaries to determine the effect of superovulation on alternation of ovarian function, two additional groups of animals were used. Group VII, consisting of four normally ovulating mice, was sacrificed at estrus and ovariectomized. The ovaries were serial sectioned and corpora lutea counted to determine the number of ova from each ovary. Group VIII, consisting of five animals, was superovulated (Gates, 1971) and sacrified on the first day following treatment. These animals were then ovariectomized and the ovaries serial sectioned and corpora lutea counted the same as group VII.

Results

The following results were observed in the three areas of the reproductive cycle studied.

Fertilization and Abortion

Twenty animals were used to determine the fertilization and abortion rates. Five mice were mated after receiving no treatment and served as the controls. Three of the five control animals (60.0%) were fertilized (see Table I). Fifteen mice were superovulated and mated and seven (46.7%) were fertilized. The abortion rates turned out differently than the fertilization rates. Of the three fertilized control mice, two carried to partruition and one aborted. Of the seven superovulated, fertilized mice, six aborted and only one carried to partruition (see Table II). This yielded an abortion rate of 33.3% in the control group and 85.7% in the superovulated groups.

Alternation of Ovarian Function

The sectioned ovaries from twelve mice were examined and corpora lutea counted to determine if any alternation of ovarian function occured. The six control mice showed a distinct right or left preference. Four mice exhibited a right preference with approximately a 2:1 ratio (see Table III). The remaining two control mice showed a left preference with approximately a 3:1 ratio (see Table III). The superovulated mice on the other hand exhibited almost exactly a 1:1 ratio from left to right (see Table III) except for one mouse with a 4:1 ratio.

Normalization of the Estrus Cycle

Resumption of normal cycling, the third area of the reproductive cycle studied, was noted upon the appearance of diestrus or proestrus following mating or treatment. In Group I, two non-fertilized control animals were observed for resumption of their estrus cycle. One animal continued cycling uninterrupted after mating while the other resumed a normal cycle after five days.

Eight superovulated animals were not fertilized upon mating and observed for resumption of their estrus cycle. One animal never resumed cycling but died after fourteen days. Another animal continued cycling with no apparent alteration in cycling caused by treatment. Of the six remaining mice, one resumed cycling after four days, another after nine days, and the remaining four animals all resumed cycling after the tenth day. An average of 7.6 days to resumption of cycling was calculated based on this data (see Table IV).

To determine the effects of mating and treatment upon the resumption of the estrus cycle, two more groups of animals were superovulated but not put with males. In Group VI, which was given PMS and HCG but not mated, all five animals continued cycling with no apparent change caused by the treatment. In Group VII receiving only PMS, following a two to three day postestrus period, cycling continued uninterrupted much the same as it had in Group VI. Both Group VI and Group VII lacked the characteristic intermediate stage in cycling characterized by pregnant mice. This intermediate stage closely resembled metaestrus (Rugh, 1968) with a very sparse distribution of

leukocytes, cornified epithelial and squamous epithelial cells all present in the vaginal smear.

Table I Fertilization rate in normally ovulated vs superovulated *Mus musculus*.

Type of ovulation	Number of animals used	Number of animals fertilized	Fertility Rate (%)
Normal ovulation	5	3	60.0%
Superovulation	15	7	46.7%

Table II Abortion rate in normally ovulated vs superovulated *Mus musculus*.

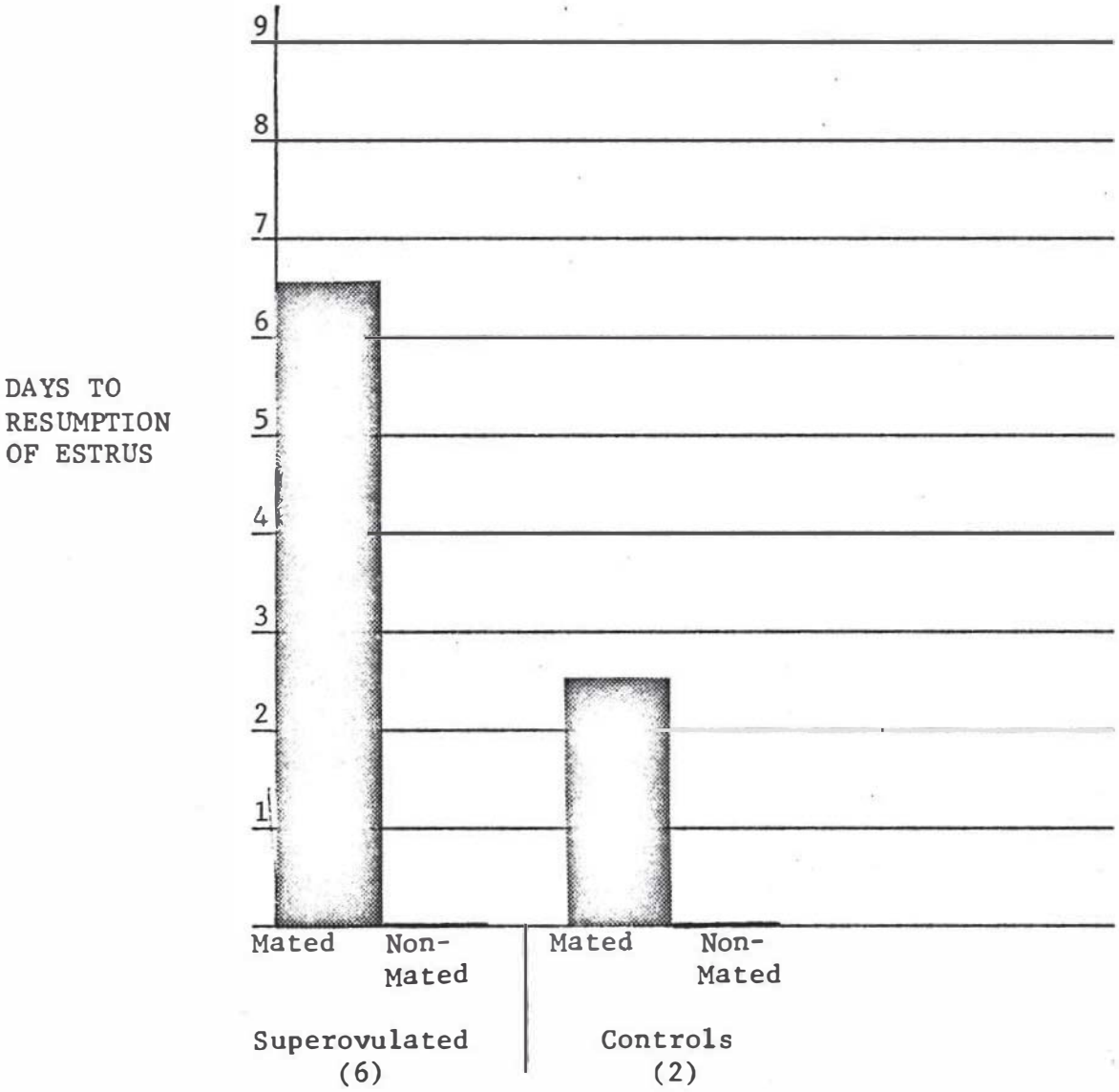
Treatment	Number of animals used	Number of animals aborted	Abortion Rate (%)
Normal ovulation	3	1	33.3%
Superovulation	7	6	85.7%

Table III. The number and percent of corpora lutea from the right and left ovaries of Mus musculus .

Controls	Left Ovary		Right Ovary		Total
	Number of CLs *	% of CLs	Number of CLs *	% of CLs	
1	9 ± 3.48	34%	17 ± 3.48	66%	26
2	10 ± 3.59	38%	16 ± 3.59	62%	26
3	8 ± 3.26	36%	14 ± 3.26	64%	22
4	5 ± 2.62	35%	9 ± 2.26	65%	14
5	26 ± 3.68	74%	9 ± 3.68	26%	35
6	19 ± 2.83	79%	5 ± 2.83	21%	24
Superovulated with PMS and HCG					
1	46 ± 6.49	51%	44 ± 6.49	49%	90
2	36 ± 5.78	51%	34 ± 5.78	49%	70
3	36 ± 5.85	50%	36 ± 5.85	50%	72
4	10 ± 3.89	19%	42 ± 3.89	81%	52
5	58 ± 7.28	52%	53 ± 7.28	48%	111
6	32 ± 5.92	43%	42 ± 5.92	57%	74

* 95% confidence interval used

Table IV The mean number of days until resumption of the estrus cycle following treatment, nontreatment, and mating.



Mus musculus studied

Discussion

Some research has been done on the fertilization rates of mice. In a study by Schneider and Hahn (1975) it was noted that mice exhibit intraspecific differences in the fertilization rates. The fertilization rate found in this experiment (46.7%) was higher than the 37.9% to 41.5% found in Schneider and Hahn's (1975) experiment, but not significantly. The differences in fertilization rates between control animals (60.0%) and superovulated (46.7%) did agree with Gates (1971), however, an increase in the ovulation rate leads to a decrease in the fertilization rate. This difference was not significant, however, using a standard t- test at the 5% level. The number of animals fertilized did appear to decrease with superovulation but not significantly.

The abortion rate was affected differently. The abortion rate increased as the number of ovulations increased. The superovulated animals aborted 85.7% of the time as compared to 33.3% of the time in the control mice. Possibly due to the small number of animals used in this experiment, no statistical significance could be shown. The results were different enough, however, to indicate that research using more animals should be done in this area to determine if a significant difference does exist.

Alternation of ovarian function is the tendency of an animal to consistently ovulate more ova from one ovary than the other. Many animals have been shown to exhibit a right or left preference (Nalbandov, 1964). In swine, 55-60% of the ova are produced from the

left ovary; in the mare, 61%. Sheep and cows on the other hand are right ovulators producing 52-59% and 60-65% respectively from the right ovary. Mice also exhibit alternation of ovarian function. Of six mice studied, four exhibited a right preference with 62-66% of the ova coming from the right ovary. The remaining two animals exhibited a left preference with 74-79% of the ova coming from the left ovary. Whether this right or left preference is alternated from left to right or is the same throughout the animal's life, as in right or left handedness, is unknown. The end result is the same, both ovaries do not ovulate the same number of ova. If the ovaries alternate in their role as primary producers of ova, then the question arises as to what regulates the ovary as a primary or secondary producer of ova at any given estrus? The regulating factor would probably be hormonal since hormones are responsible for the maturation and ovulation of follicles in each ovary. There is a possibility that mice exhibit a right or left preference throughout life. This is supported by the fact that the size of the ovary and its blood supply are approximately the same throughout the animal's mature life. Therefore, the relative percent of FSH and LH in the circulating blood reachingeach ovary would remain the same throughout the adult life of the mouse. So, a right or left preference is probably a lifelong characteristic of the animal.

Another theory has already been set forth as to how alternation of ovarian function occurs in the form of corpora lutea accessoria. Both ovaries produce a fixed number of tertiary follicles. Some of these follicles will be ovulated and the remainder are what Greenwald

(1962) referred to as "reserve" follicles, which atrophy to become CLA's. Possibly the only difference between these reserve follicles and the ovulated follicles is the level of endogenous LH available at estrus to ovulate them. In the ovary which ovulated the greater number of ova there should be a greater blood supply. More follicles matured in the preferred ovary indicates a greater FSH concentration than the other ovary and thus a greater blood supply. Since the blood supply to each ovary is approximately the same throughout the animal's life, this relative balance would remain the same. Thus the animal would always mature and ovulate more follicles from the ovary with the richest blood supply. The blood supply would also seem to be the determining factor as far as which follicles are ovulated within each ovary. Those follicles with the first, most direct, supply of blood would also have the first supply of available FSH and LH. This would cause them to mature and eventually be ovulated while the "reserve" follicles receive only an insufficient surplus of the critical hormones. Since many of the follicles are not ovulated, LH must be the critical factor and in smaller supply. Those follicles not ovulated evidently did not receive enough LH. Many other factors besides blood supply and hormone level could be involved but none seems as important at this time. Much more research in this area is still needed, however, to determine exactly what factors do influence alternation of ovarian function.

Superovulation supplies FSH and LH in large enough doses to cause the reserve follicles to mature and be ovulated. Even though the blood supply remains the same to both ovaries, the critical hormones are now provided in large enough concentrations to supply all of the available

follicles. Since both ovaries are capable of producing the same number of ova (see Table III) with superovulation the reason they do not must be in the distribution and concentration of hormones to each ovary. Superovulation overrides the natural ovarian preference by making available large amounts of the critical hormones.

The superovulated animal having only 10 corpora lutea in the left ovary as opposed to 42 in the right, contrasted sharply with four of the remaining five animals which exhibited almost exactly a 1:1 ratio from left to right ovary. Due to the comparative regularity of the other five mice, as opposed to the sharply contrasting results of the sixth mouse, other factors were thought responsible. What these other factors are or how they influence individual animals are questions which will have to be answered by future research in the area. The usual 1:1 ratio of left to right ova following superovulation suggests that no alternation of ovarian function occurs following superovulation treatment.

No significant difference was noted at the 5% level between the mean of 2.5 days to resumption of cycling for the control mice and the mean of 7.6 days for the superovulated animals. This was possible due to the small number of animals involved. An interesting thing, however, was found when comparing the average time to resumption of cycling following superovulation with mating and superovulation without mating. Both of the groups which were superovulated, but not mated, continued cycling apparently unaffected by treatment. However, when treatment was combined with mating, a very significant difference was noted at the 5% level. This indicates that mating caused a delay in resumption of cycling even if fertilization did not occur. This delay varies in

length for different animals, but animals receiving both treatment and mating showed a significantly longer delay before resuming the normal cycling than do treated animals that have not been mated. The delay ranged from 0-10 days in the animals studied. The reason for this delay in resumption of cycling is unknown. One possible explanation might be a change brought about by the introduction of seminal secretion from the male upon copulation much the same as a copulation plug (Rugh, 1968). Whatever the cause, the result is the same: a delay in the cycling and a longer quiescent period until the next mating.

Summary

In the first area studied, no significant differences were noted between the fertilization rates or abortion rates in superovulated as opposed to control mice. However, research using more animals needs to be done in this area to prove this conclusively. In the second area studied, alternation of ovarian function is exhibited in control mice with some showing a left preference and some a right. Superovulated mice exhibited less alternation probably due to the resulting maturation and ovulation of the "reserve" follicles (Greenwald, 1962). In the third area of this study mated animals exhibited a significantly longer delay than non-mated animals in the resumption of cycling in both superovulated and control mice. This is possible due to seminal secretions introduced as a result of mating.

Literature Cited

Beaumont, H. M. and A. F. Smith. 1975. Embryonic Mortality During the Pre- and Post-implantation periods of pregnancy in mature mice after superovulation. J. Reprod. Fertil. 45(3): 437-448.

Biggers, J. D., W. K. Whiten, and D. G. Whittingham. 1971. The Culture of Mouse and Embryos in Vitro. Methods in Mammalian Embryology: 86-112.

Chretien, F. C. 1972. Long term effects of superovulatory treatment on gestation in rats. Ann. Endocrinol. 33(5): 507-516.

Fengold, S. M., and E. W. Hahn. 1972. Postconception development of rat ova following x-ray induced superovulation. Radiat. Res. 51(1): 110-120.

Fujimoto, S., N. Pahlovan, and W. R. Dukelow. 1974. Chromosome abnormalities in Rabbit Pre-implantation Blastocysts induced by Superovulation. J. Reprod. Fertil. 40(1): 177-181.

Gates, A. H. 1971. Minimizing Yield and Developmental Uniformity of Eggs. Methods in Mammalian Embryology: 64-74.

Greenwald, G. S. 1962. Analysis of Superovulation in the Adult Hampster. 71(3); 378-389.

Greenwald, G. S. 1963. Effects of an anti PMS serum on superovulation. The Ana. Rec. 145(2): 238.

Hafez, E. S. E. 1964. Seasonal fluctuation in Ovulation rate and Superovulatory response of the Domestic Rabbit. ACTA Zoologica 45(½): 123-131.

Hafez, E. S. E. 1969. Superovulation and preservation of mammalian egg. ACTA Endrocrinol. Suppl. 62(140): 5-44.

Ikeda, H. and Y. Sakuma. 1974. Studies on increase of litter size in hamsters. Bull. Co. Agric. Vet. Med. Nikon Univ. 31. 282-290.

Ishijima, Y. and K. Ishida. 1970. Histological studies of rabbit ovaries with superovulation treatment. Jap. J. Fert. Steril. 15(3): 194-200.

Ishijima, Y., M. Ito, T. Hirabayashi, and Y. Sakuma. 1971. Effect of administration of prolactin on pregnancy in rabbits after superovulation. Jap. J. Fert. Steril. 16(1): 1-5.

Ishijima, Y., Y. Kawase, T. Hirabayshi. 1971. Effect of superovulation on the tubal transport and cleavage rates of ova in mice. J. Agric. Sci. Tokyo Nogyo Daigaku. (Spec. issue): 63-72.

Ishijima, Y., H. Mihata, T. Hirabayashi, Y. Sakuma. 1971. Effect of Estradiol on superovulation in adult hamsters. J. Agric. Sci. Toyko Nogyo Diagaku. 16(1): 49-51.

Lang, D. R. and D. R. Lamond. 1966. Some factors affecting the response of the immature mouse to PMS and HCG. J. Endo. 34(1): 41-50.

Mandl, A. M. 1964. Superovulation following ovarian x-irradiation. J. Reprod. Fertil. 8(3): 375-396.

Maurer, R. R., W. L. Hunt, and R. H. Foote. 1968. Repeated super-ovulation following administration of exogenous gonadotropins in Dutch-belted rabbits. J. Reprod. Fertil. 15(1): 93-102.

Maurer, R. R. and R. H. Foote. 1971. Maternal aging and embryonic mortality in the rabbit. L Repeated superovulation, embryo culture and transfer. J. Reprod. Fertil 25(3): 329-341.

Moore, P. J., and G. S. Greenwald. 1974. Seasonal variation in ovarian responsiveness of the cycling Hamster in PMSG. J. Reprod. Fertil. 36(1): 219-220.

Nalbandov, A. V. 1964. Reproductive Physiology. W. H. Freeman and Co. San Francisco and London. 316 pp.

Rugh, R. 1968. The Mouse, Its Reproduction and Development. Burgess Pub. Co. Minneapolis, Minn.

Sakuma, Y. and T. Endo. 1970. Studies on the superpregnancy and superfecundation of the superovulated mice with dosage of prolactin. I. Jap. J. Fert. Steril. 15(1): 1-6.

Sato, A. 1962. Induction of superovulation and pregnancy in mature and immature rats by the application of gonadotropins. Jap. Journ. Genet. 37(3): 253-259.

Schneider, U. and J. Hahn. 1975. Results of in vitro fertilization experiments with mouse ova under consideration of different breeds, different hormone levels for superovulation and different amounts of BSA in the fertilization medium. Z. Versuchsteirkd 17(1): 27-34.

Weifenbach, H. 1965. The influence of FSH, HCG, and progestational substances on the compensatory superovulation in the rat. ACTA Endocrinologica 50(1): 7-14.

Wilson, E. D., M. N. Runner, and M. X. Zarrow. 1963. Effect of hypothyroidism, diet and litter size on superovulation in the mouse and rat. J. Reprod. Fertil. 5(2): 233-238.

Wyss, H. I., and G. Pincus. 1964. Effect of PMSG, Estradiol and Progesterone on Superovulation in the immature rate. Endocrino. 75(4): 586-591.

Zarrow, M. X., and D. L. Quinn. 1963. Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS-induced superovulation. Jour. Endocrinol. 26(2): 181-188.